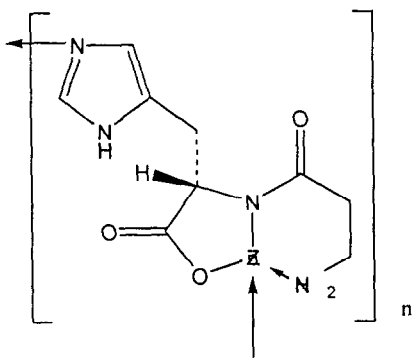
Specifications and Test Methods of ZincCarnosine

Hamari Chemicals, Ltd.

□. **Substance**

(1) Name: ZincCarnosine

(2) Structural formula, molecular formula, and molecular weight



$(C_9H_{12}N_4O_3Zn)_n$ 289.61×n

□.**Specifications**

No.	Test Name	Specification
1	Appearance	White to pale yellowish white crystalline powder
2	Odor	Odorless
3	Identification	Positive
4	Optical rotation	$[\ ]_D^{20}$ 8 9
5	Clarity and color of solution	Clear and colorless or not more than the control solution
6	Related substances	NMT 1.0%
7	Water	NMT 5.0%
8	L-Carnosine	76.0 80.0%
9	Zinc	21.5 23.0%

□. **Standard test**

Unless otherwise specified, use general notices and general tests of The Pharmacopoeia of Japan.

□. **Storage:** Preserve tight containers.

□. **Test methods**

1. Appearance: ZincCarnosine occurs as white to pale yellowish white crystalline powder.

2. Odor: ZincCarnosine is odorless.

3. Identification: Positive

Determine the infrared absorption spectrum of ZincCarnosine as directed in the potassium bromide disk method under the Infrared Spectrophotometry: it exhibits absorption at the wave numbers of about 3280 cm^{-1}, 1622 cm^{-1}, 1559 cm^{-1}, 1386 cm^{-1}, 1260 cm^{-1}, 1115 cm^{-1} and 999 cm^{-1}.

4. Optical rotation : $[\ ]_D^{20}$ 8 9 (0.4 g calculated as the anhydrous basis, 3 mol/L Hydrochloric acid TS, 20 mL, 100 mm)

5. Clarity and color of solution: Clear and colorless or not more than the control solution

Dissolve 0.2 g of ZincCarnosine in 20 mL of dilute hydrochloric acid: the solution is clear and colorless or the turbidity is not thicker than that of the control solution.

Control solution: To 1.0 mL of the standard solution add water to make 20 mL, and add 1 mL of diluted nitric acid (1 3), 0.2 mL of a solution of dextrin(1 50) and 1 mL of a solution of silver nitrate(1 50), and allow to stand for 10 minutes.

Standard solution: To 14.1 mL of 0.1 mol/L hydrochloric acid add water to make exactly 50 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL.

6. Related substances: Not more than 1.0 %

Dissolve 0.050 g of ZincCarnosine in 10 mL of 0.1 mol/L hydrochloric acid TS, add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 5 L each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of L-Carnosine from the sample solution is not larger than the peak area of L-Carnosine from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm)

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography(5 m in particle diameter).

Column temperature: A constant temperature of about 45 C

Mobile phase: Dissolve 1.4 g of potassium dihydrogenphosphate in water to make 1000 mL, and adjust the pH to 3.5 with diluted phosphoric acid. Dissolve 2 g of sodium 1-octanesulfonate in 900 mL of this solution and add 100 mL of acetonitrile.

Floe rate: Adjust the flow rate so that the retention time of L-Carnosine is about 15 minutes.

Selection of column: Dissolve 0.05 g of L-Carnosine and 0.05 g of L-Histidine in 10 mL of 0.1 mol/L hydrochloric acid TS, add the mobile phase to make 100 mL. Proceed with 5 L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of L-Histidine and L-Carnosine in this order with the resolution between these peaks being not less than 7.

Detector sensitivity: Adjust the detection sensitivity so that the peak height of L-Carnosine obtained from 5 L of the standard solution is 3 10 mm.

Time span of measurement: About 4 times of the retention time of L-Carnosine.

7. Water: Not more than 5.0% (0.2 g, volumetric titration, direct titration)

8. L-Carnosine: 76.0 80.0 %

Weigh accurately about 0.1 g of ZincCarnosine, dissolve in 80 mL of acetic acid(100) by warming, cool, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L Perchloric acid 1 mL VS = 11.312 mg of $C_9H_{14}N_4O_3$

9. Zinc: 21.5 23.0 %

Weigh accurately about 0.2 g of ZincCarnosine , dissolve in 3 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet 25 mL of this the solution, add 10 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g eriochrome black T-sodium chloride indicator).

Each mL of 0.01 mol/L disodium dihydrogen
ethylenediamine tetraacetate VS
= 0.6539 mg of Zn